

We Claim

1. A process for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a group of fragments, said method comprising:

a) preparing an initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;

b) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries;

c) screening said group of monodigested libraries for said known characteristic to detect the presence of intact target fragments, to thereby determine those restriction enzymes to which said target fragment is insensitive; and

d) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multidigested library having an intact clone of the target nucleic acid fragment.

2. The method of claim 1 wherein said plurality of restriction enzymes comprises at least 10 restriction enzymes.

3. The method of claim 2 wherein said plurality of restriction enzymes comprises at least 50 restriction enzymes.

4. The method of claim 3 wherein said plurality of restriction enzymes comprises at least 70 restriction enzymes.

5. The method of claim 1 wherein said predetermined number of known restriction sites is four.

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6. The method of claim 1 wherein said predetermined number of known restriction sites is three.

7. The method of claim 6 wherein at least one of said three sites is different from, and flanked by, said two remaining sites.

8. The process of claim 1 wherein said restriction enzymes have cleavage sites from 5 to 6 nucleotides in length.

9. The method of claim 1 including the further step of transforming and replicating said intact clone of the target nucleic acid fragment.

10. The method of claim 9 including the further step of isolating said intact clone.

11. The method of claim 9 including the further steps of cleaving, purifying, and sequencing said fragment.

12. The method of claim 1 comprising, after step b), the further step of transfecting said monodigested libraries in cellular hosts;

13. The method of claim 1 comprising the further step of verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment.

14. The method of claim 1 comprising the further step of verifying the presence of said target fragment in said multi-digested library by transforming said library and screening said transformed library for the presence of said target fragment.

15. The method of claim 1 wherein said group of fragments contains from 1 to 108 fragments, each from about 0.1 kb to 5 kb in size.

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16 A method for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a group of fragments, said method comprising:

a) preparing an initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;

b) verifying the presence of said target fragment in said initial library by transfecting in a cellular host and screening said transfected host for the presence of said target fragment;

c) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries;

d) independently transfecting said monodigested libraries;

e) screening said transfected monodigested libraries for said known characteristic to detect the presence of intact target fragments, to thereby determine those restriction enzymes to which said target fragment is insensitive;

f) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multidigested library having an intact clone of the target nucleic acid fragment; and

g) transforming said multidigested library.

17. The process of claim wherein said restriction enzymes have cleavage sites from 5 to 6 nucleotides in length.

18 A process for isolating an intact clone of one target nucleic acid fragment having a known characteristic, from a group of fragments, said method comprising:

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a) preparing an initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;

b) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of mono-digested libraries;

c) transforming said monodigested libraries into bacteria;

d) culturing said bacteria to produce digested libraries substantially free of cleaved products, cleaving each digested library to produce digestion products, depositing said products in an agarose gel well, migrating said products, transferring said products onto a membrane, hybridizing said transferred products with a probe, to thereby determine those restriction enzymes to which said target fragment is insensitive; and

d) subjecting said initial library to substantially all of said plurality of restriction enzymes to which said target fragment is insensitive, to produce a multi-digested library having an intact clone of the target nucleic acid fragment.

19. A method for producing a series of monodigested libraries from a group of fragments, said method comprising:

a) preparing an initial library of clones from said group of fragments using a vector containing no more than a predetermined number of known restriction sites;

b) subjecting said initial library to a plurality of restriction enzymes individually, which plurality of enzymes do not include those to which said vector is sensitive, to produce a group of monodigested libraries;

20. The group of monodigested libraries produced by the process of claim 19.

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